In vivo ESR determination of intracellular oxidative stress by acyl-protected hydroxylamine

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Acyl-protected hydroxylamines as new spin reagents to make ESR measurements of intracellular oxidative stress have been developed [1]. These spin reagents are stable non-radical compounds that are not affected by oxidation outside cells because of acyl-protection. However, inside cells, they are easily deprotected with intracellular esterase to yield hydroxylamines, which are oxidized by oxidative stress (such as the production of active oxygens species) to yield ESR-detectable stable nitroxide radicals. On the basis of EPR signal intensity of this radical, intracellular oxidative stress can be estimated. In the in vitro ESR study, it was confirmed that the sensitivity of one of acyl-protected hydroxylamines, 1-acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrrolidine (ACP), was about 10 times higher than that of a conventional spin trapping reagent in human leukocytes [1]. On the other hand, an in vivo ESR spectrometer operating at ca. 700 MHz was developed to detect radicals in the small animals such as rats and mice [2,3]. By using ACP and the in vivo ESR spectrometer, estimation of intracellular oxidative stress was performed in some disease models. In a kainic acid-induced seizure of rats, it was found that the oxidative stress in the hippocampus and striatum was enhanced, but not so in the cerebral cortex [4]. In a puromycin-induced nephrosis of rats, it was found that the renal oxidative stress was augmented 1 hour after the injection of puromycin.

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